SUPPLEMENTARY EXPERIMENTAL PROCEDURES

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. NLRP3 and AIM2 inflammasomes provide host resistance to *Aspergillus* infection. Related to Figure 1.

(A) Survival of WT and *Aim2*$^{-/-}$*Nlrp3*$^{-/-}$ mice infected with 1×10^5 *A. fumigatus* conidia in the absence of immunosuppression with cyclophosphamide and cortisone acetate.

(B) Body weight change of WT and *Aim2*$^{-/-}$*Nlrp3*$^{-/-}$ mice infected with 1×10^5 *A. fumigatus* conidia in the absence of immunosuppression with cyclophosphamide and cortisone acetate.

WT, *Nlrp3*$^{-/-}$, *Aim2*$^{-/-}$, *Aim2*$^{-/-}$*Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ mice were infected with 1×10^5 *A. fumigatus* conidia after immunosuppression with cyclophosphamide and cortisone acetate.

(C) Lung tissue sections were stained with Gomori Methenamine Silver (GMS). Quantification of hyphal dissemination in the lung tissues.

(D) Lung tissue sections were immunostained with myeloperoxidase (MPO). Quantification of the number of MPO-positive cells in the lung tissues. Tukey's multiple comparison test, ****, P<0.0001; ns, not statistically significant.

Figure S2. Bone marrow and stromal compartments contribute to the host defense against *Aspergillus* infection. Related to Figure 2.

Bone marrow chimeras were infected with 1×10^5 *A. fumigatus* conidia after immunosuppression with cyclophosphamide and cortisone acetate.

(A) Lung tissue sections were stained with GMS. Quantification of hyphal dissemination in the lung tissues.

(B) Lung tissue sections were immunostained with MPO. Quantification of the number of MPO-positive cells in the lung tissues. Tukey's multiple comparison test, *, P<0.05; **, P<0.01; ****, P<0.0001.

Figure S3. Inflammasome activation is induced by *Aspergillus* infection. Related to Figure 3.

(A-B) Bone marrow-derived dendritic cells (BMDCs) from WT mice were infected with *A. fumigatus* (MOI 20), *C. albicans* (MOI 5) for 20 h or stimulated with LPS and silica for 8 h, LPS and ATP for 30 min, LPS and nigericin for 45 min or transfected with poly(dA:dT) for 5 h. Cell lysates were analyzed for caspase-1 activation and levels of IL-1β release.

(C and D) Unprimed or LPS-primed WT BMDCs were infected with *A. fumigatus* for 20 h. Caspase-1 activation was analyzed from the cell lysate and levels of IL-1β released were analyzed from the supernatant.

(E and F) Unprimed BMDCs from WT, *Nlrp3*$^{-/-}$, *Nlrc4*$^{-/-}$, *Aim2*$^{-/-}$, and *Nod1*$^{-/-}$ mice were infected with *A. fumigatus* for 20 h. Caspase-1 activation was analyzed from the cell lysate and levels of IL-1β released were analyzed from the supernatant.

(B, D and F) Data represent means ± SEM of triplicate wells. Data are representative of three or more independent experiments. Tukey's multiple comparison test, **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, not statistically significant.

Figure S4. Both NLRP3 and AIM2 are required for inflammasome activation in response to *Aspergillus* infection. Related to Figure 3.

(A-C) BMDCs from WT, *Nlrp3*$^{-/-}$, *Aim2*$^{-/-}$ *Nlrp3*$^{-/-}$*Aim2*$^{-/-}$ and *Asc*$^{-/-}$ mice were infected with *A. fumigatus* (MOI 20) for 20 h. Caspase-1 p20 released into the supernatant were analyzed for caspase-1 activation. The levels of IL-18, TNF-α, KC and IL-6 released were analyzed from the supernatant.

(D) The number of conidia phagocytosed by or attached to BMDCs after 1 h of infection was used to calculate the % phagocytosis and phagocytic index. The number of conidia phagocytosed by or attached to BMDCs after 6 h of infection was normalized to the number of conidia phagocytosed or attached after 1 h of infection to determine the level of intracellular conidia killing. Data represent means ± SEM of triplicate wells. Data are representative of three or more independent experiments.

(B) Tukey's multiple comparison test, **, P<0.01; ns, not statistically significant.

Figure S5. *Aspergillus* DNA activates the AIM2 inflammasome and phagocytosis is required for inflammasome activation in response to *Aspergillus* infection. Related to Figure 3.

(A and B) BMDCs from WT, *Nlrp3*$^{-/-}$, *Aim2*$^{-/-}$ *Nlrc4*$^{-/-}$ and *Asc*$^{-/-}$ mice were infected with *A. fumigatus* (MOI 20) for 20 h. Caspase-1 activation was analyzed from the cell lysate and levels of IL-18 released were analyzed from the supernatant.

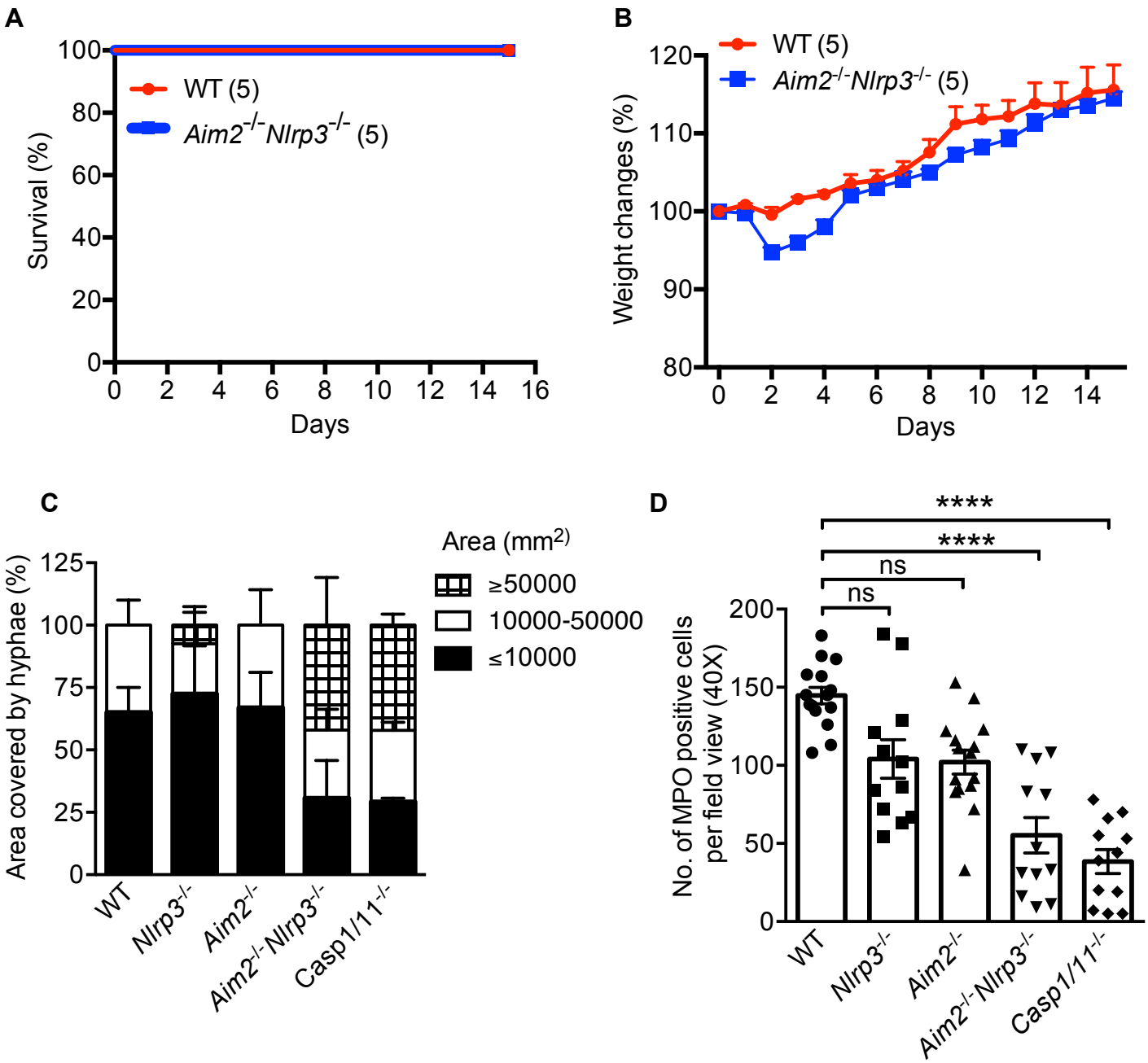
(C and D) WT BMDCs were infected with *A. fumigatus* (MOI 20) for 20 h in the absence or presence of 5 or 50 μM of cytochalasin B or cytochalasin D. Caspase-1 activation was analyzed from the cell lysate and levels of IL-1β released were analyzed from the supernatant.

Data represent means ± SEM of triplicate wells. Data are representative of two independent experiments.

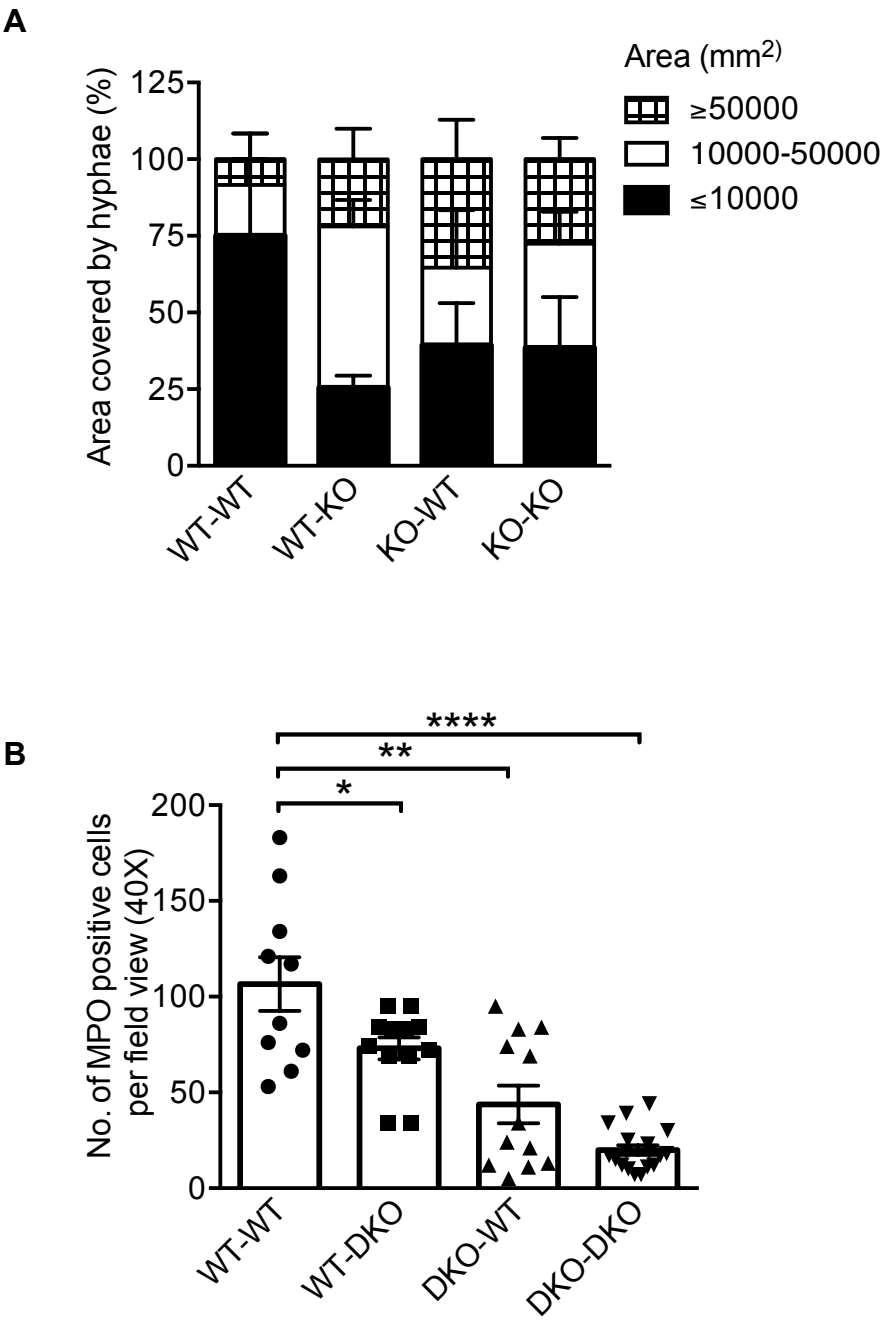
(B and D) Tukey's multiple comparison test, **, P<0.01; ***, P<0.001.

Figure S6. Levels of pro-inflammatory cytokines in WT and *Aim2*$^{-/-}$*Nlrp3*$^{-/-}$ mice were similar after *Aspergillus fumigatus* infection. Related to Figure 1.

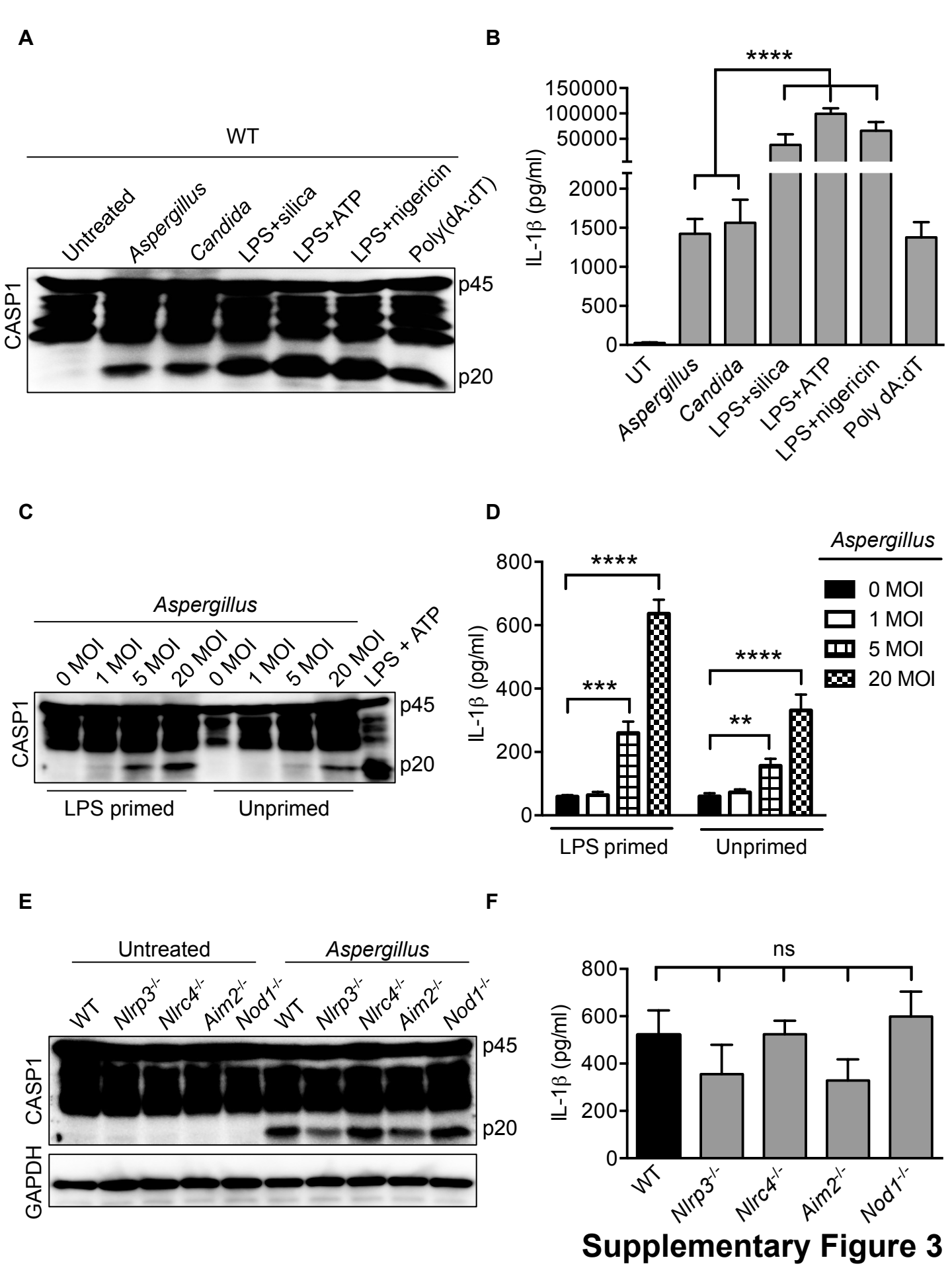
(A-C) Levels of IL-6, KC and TNF-α in lung homogenates were similar between WT and *Aim2*$^{-/-}$*Nlrp3*$^{-/-}$ mice after 3 days of infection with *A. fumigatus*. Unpaired t-test, ns, not statistically significant.



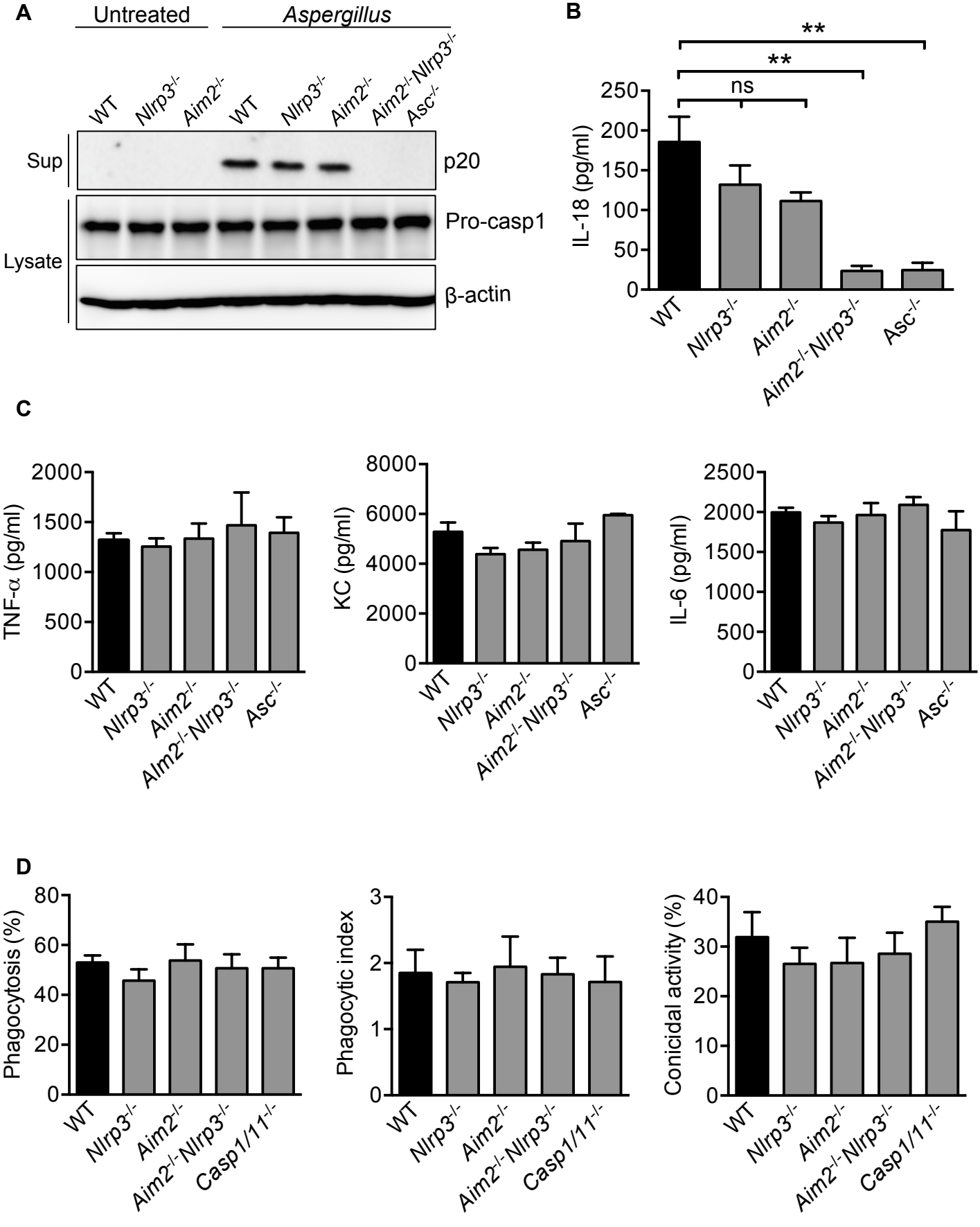
Supplementary Figure 1



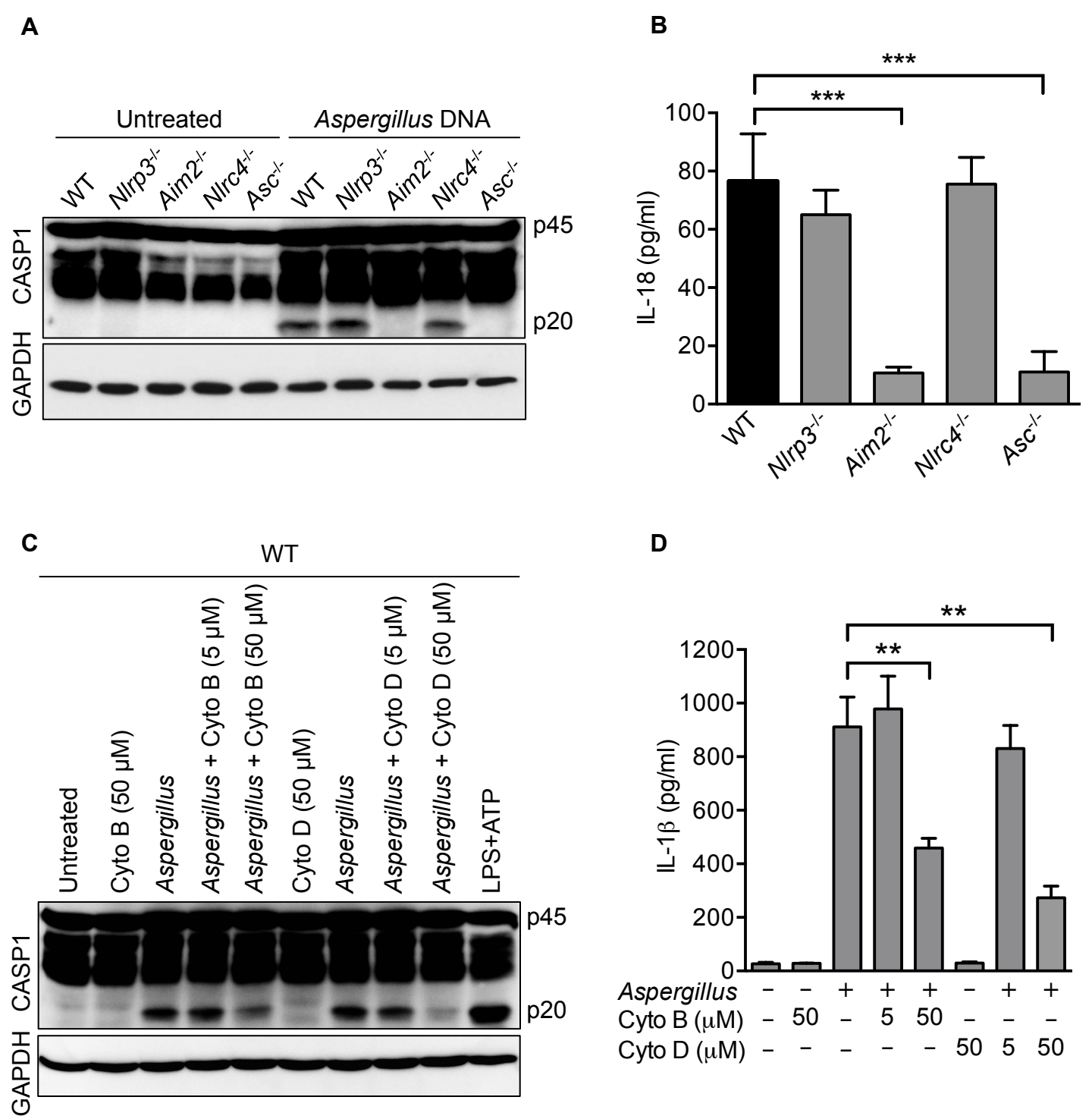
Supplementary Figure 2



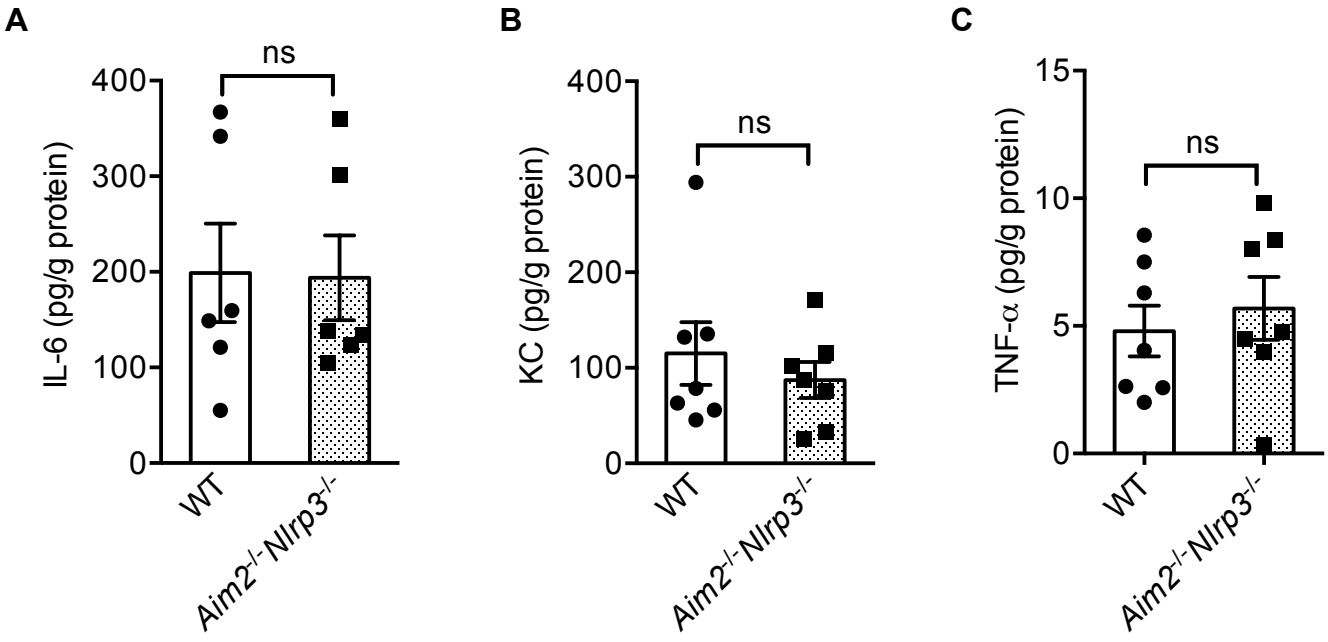
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6